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1 **Title**

2 The RNA binding protein La/SSB promotes RIG-I-mediated type I and type III IFN
3 responses following Sendai viral infection

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4

5 **Conflict of Interest Statement**

6 The authors have no financial conflict of interest with the work and results
7 presented herein.

8

9

1 **Abstract**

2 La/SS-B (or La) is a 48 kDa RNA-binding protein and an autoantigen in autoimmune
3 disorders such as systemic lupus erythematosus (SLE) and Sjögren's syndrome
4 (SS). La involvement in regulating the type I interferon (IFN) response is
5 controversial - acting through both positive and negative regulatory mechanisms;
6 inhibiting the IFN response and enhancing viral growth, or directly inhibiting viral
7 replication. We therefore sought to clarify how La regulates IFN production in
8 response to viral infection. ShRNA knockdown of La in HEK 293T cells increased
9 Sendai virus infection efficiency, decreased IFN- β , IFN- λ 1, and interferon-stimulated
10 chemokine gene expression. In addition, knockdown attenuated CCL-5 and IFN- λ 1
11 secretion. Thus, La has a positive role in enhancing type I and type III IFN
12 production. Mechanistically we show that La directly binds RIG-I and have mapped
13 this interaction to the CARD domains of RIG-I and the N terminal domain of La. In
14 addition, we showed that this interaction is induced following RIG-I activation and
15 that overexpression of La enhances RIG-I-ligand binding. Together, our results
16 demonstrate a novel role for La in mediating RIG-I-driven responses downstream of
17 viral RNA detection, ultimately leading to enhanced type I and III IFN production and
18 positive regulation of the anti-viral response.

19

20

1 Introduction

2 Host viral detection systems rely mostly on recognition of viral nucleic acids by
3 pattern recognition receptors (PRRs) including, RNA and DNA-sensing Toll-like
4 receptors (TLR-3, -7, -8, -9), DNA receptors (DAI, AIM2, IFI16, DDX41) and RIG-I-
5 like receptors (RLRs). RIG-I is an essential type I and type III IFN-inducing receptor
6 required for the detection of negative-sense single stranded RNA viruses such
7 Sendai virus, a member of the *Paramyxoviridae* family, in addition to *Rhabdoviridae*
8 and *Orthomyxoviridae* family members^{1,2,3}. Upon recognition of pathogenic RNA, an
9 ATP-dependent conformational change is triggered in RIG-I exposing the activatory
10 CARD domains. This allows interaction between the second CARD domain of the
11 receptor and the CARD domain of downstream mitochondrial-associated adaptor,
12 IPS-1⁴⁻⁶. This interaction leads to assembly and activation of downstream IKK-
13 related kinases TBK-1 and IKK- ϵ , that subsequently phosphorylate IRF-3 and IRF-7
14^{7,8}. This ultimately results in transcriptional induction of both type I and type III IFNs,
15 which in turn leads to robust expression of IFN-stimulated genes (ISGs)^{9,10}.

16 Type I IFNs, including IFN- α , - β , - ω , - κ and - ϵ , act on cells via binding to the IFN-
17 α receptor (IFN α R), comprised of an IFN α R1 and IFN α R2 heterodimer^{11,12}. Type I
18 IFN synthesis occurs in virtually all cell types downstream of anti-viral PRR
19 recognition of viral RNA/DNA. Once secreted by the virally-infected cell, type I IFNs
20 bind and activate IFN α R, leading to induction of interferon stimulated genes (ISGs)
21 through activation of JAK1 and Tyk2, followed by phosphorylation of signal-
22 transducing activators of transcription (STAT) proteins STAT1 and STAT2¹³⁻¹⁶.
23 ISGs, including RIG-I, TLR-3, OAS1 and OAS2, are expressed following
24 STAT1/STAT2 activation, leading to the inhibition of transcription and translation of
25 viral proteins^{17,18}, along with induction and synthesis of MHC class I expression.
26 This makes the cell more susceptible to CD8⁺ cytotoxic T cells^{19,20}, activates NK
27 cells which selectively kill virus-infected cells^{21,22}, and leads to maturation of DCs²³
28 and B cell responses^{20,24}.

29 Functional members of the Type III IFN family, including IFN- λ 1 (IL-29), IFN-
30 λ 2 (IL-28A) and IFN- λ 3 (IL-28B), are induced downstream of TLR-3 and RLR
31 signalling^{25,26} but signal through an independent cell-surface receptor complex,
32 consisting of IL10R2 (also called CRF2-4) and IFN- λ R1 (also called IL-28RA)^{27,28}.
33 While the type I IFN receptor is ubiquitously expressed, the expression of the IFN-

1 AR1 component of the type III IFN receptor complex appears to be more limited and
2 restricted to cells of epithelial origin, plasmacytoid DCs, macrophages, monocyte-
3 derived DCs and intra-hepatic natural killer cells (NKs) ²⁹. Upon type III IFN binding
4 to the receptor, a signal transduction cascade ensues involving activation of JAK1,
5 JAK2 and Tyk2, followed by STATs activation and ISG expression, almost identical
6 to that induced by type I IFN receptor ^{27,30}.

7 Whilst anti-viral TLRs and RLRs are well recognised for their role in inducing
8 type I and type III IFNs, more recently RNA polymerase III (RNA pol III), an enzyme
9 involved in the transcription of non-coding RNA, was reported to act as an anti-viral
10 PRR by regulating type I IFN induction through generation of a RIG-I ligand ^{31,32}.
11 RNA pol III is able to transcribe AT-rich dsDNA into the 5'ppp-dsRNA format required
12 for recognition by RIG-I and subsequent IFN induction ³². Interestingly, an
13 autoantigen associated with systemic autoimmune disease, La/SSB (La), binds to
14 RNA pol III transcripts and stabilises newly-synthesised RNAs ³³⁻³⁸. In addition to its
15 interaction with a large variety of newly-formed RNAs, La binds a number of virus-
16 encoded RNAs, such as adenovirus VA RNA I and VA RNA II, EBV EBER 1 & 2
17 RNA, and leader RNA of negative strand RNA viruses ³⁹⁻⁴². Because La can interact
18 with viral RNA, studies have sought to clarify its role in anti-viral immunity. Some
19 studies proposed that La is manipulated by viruses in an attempt to block the anti-
20 viral response, which it reportedly achieves by binding and sequestering the dsRNA
21 ligand for RIG-I, thus preventing activation of the pathway ⁴³⁻⁴⁵. On the other hand,
22 La was also shown to promote an anti-viral response to *flock house* virus (FHV),
23 although the mechanism involved was unclear ⁴⁶. Thus the role of La in regulating
24 anti-viral immune responses is not well understood.

25 Our work described herein demonstrates a novel positive role for La in
26 regulating type I and type III IFN responses downstream of Sendai virus infection.
27 Our results show that knockdown of La severely impairs the ability of cells to mount
28 an anti-viral response to Sendai virus infection, resulting in enhanced infectivity, as a
29 result of reduced type I and III IFN production. We observed that La bound RIG-I in a
30 ligand-inducible manner and that the CARD domains of RIG-I and RNA-binding
31 domain of La are required for this interaction. The association between La and RIG-I
32 promotes the interaction of RIG-I with dsRNA, thereby enhancing RIG-I-driven type I
33 and type III IFN induction. Thus, La is required for an optimum IFN response to

- 1 Sendai virus infection by binding to the anti-viral RIG-I receptor and promoting its
- 2 interaction with its cognate ligand.

3

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5

1 **Results**

2 ***La depletion results in enhanced Sendai virus infection efficiency and*** 3 ***decreased Type I and Type III Interferon responses***

4 Sendai virus (SeV) strains are enveloped paramyxoviruses with single-stranded,
5 non-segmented, negative sense RNA genomes. SeV strains may vary significantly in
6 their degrees of virulence. A number of virulence factors have been mapped to either
7 structural proteins associated with differential virus attachment and entry into host
8 cells, or non-structural proteins implicated in immune modulation that includes
9 antagonism of interferon signalling ⁴⁷⁻⁵⁰. This study utilized a recombinant SeV
10 expressing eGFP (rSeV/eGFP) and SeV *Cantell* strain, both capable of inducing type
11 III IFN responses, while only the *Cantell* strain induces an additional robust type I
12 IFN response ^{51,52}. Evidence suggests that this differential induction of type I IFN is
13 due to the presence of defective-interfering (DI) particles in the *Cantell* strain ^{53,54}.

14 To investigate the effect La knockdown on Sendai infectivity, HEK 293T cells
15 infected with rSeV/eGFP and transfected with either a La-specific or scrambled
16 shRNA were evaluated by UV microscopy, using areas of comparable monolayer
17 confluency for image analyses (Figure 1). Supplemental Figure 1 demonstrates
18 successful La depletion in HEK 293T cells, both at gene (Supplemental Figure 1a &
19 b) and protein (Supplemental Figure 1c & d) levels, validating the La shRNA
20 construct used throughout this work. Analysis of fluorescence (using Image J
21 software) demonstrated higher eGFP coverage following rSeV/eGFP infection in
22 cells depleted of La compared with those transfected with the scrambled control,
23 suggesting that knockdown of La enhanced viral infectivity (Figure 1a and b).
24 Importantly, the increased eGFP coverage seen in La-depleted monolayers was
25 reflected in an increase in viral titres released from these cells, compared with
26 control cells (Figure 1c). As La has been published to be involved in a number of
27 cellular processes, including RNAi processing, the viability of cells transfected with
28 scrambled or La shRNA was compared in order to ensure that La knockdown did not
29 affect cell viability. As shown in Figure 1d, cell viability was equivalent across the 2
30 different experimental conditions.

31 We next investigated the effect of La knockdown in HEK 293T cells on type I
32 and type III IFN induction by quantitative PCR (qPCR) following rSeV/eGFP or SeV

1 *Cantell* infection. As stated above, rSeV/eGFP induced a strong type III IFN
2 response but no type I IFN, whereas the SeV *Cantell* induced a robust type I IFN
3 response in addition to type III IFNs^{51,52}. La knockdown resulted in significant
4 reduction of both IFN- β and IFN- λ 1 mRNA levels following SeV *Cantell* infection
5 (Figure 2a and b), whereas a reduction in only IFN- λ 1 expression was observed in
6 La-depleted cells following infection with rSeV/eGFP, albeit at the later time point of
7 48 hours post-infection (hpi) (Figure 2e and f). Importantly, La knockdown had no
8 effect on housekeeping gene expression as shown in supplemental Figure 1e.
9 Interestingly, expression of CXCL-10 (IP-10) was significantly attenuated by La
10 depletion following infection with both SeV strains, compared with scrambled
11 controls (Figure 2d and h). CXCL-11 was only impaired in La-depleted cells following
12 *Cantell* infection (Figure 2c and g), suggesting that CXCL-11 induction is specifically
13 regulated by La in the context of a type I IFN response. In contrast, the induction of
14 CXCL-11 in response to rSeV/eGFP was poor, with La depletion having no effect on
15 the cells' ability to mount a response.

16 Analysis of cytokine release following SeV infection demonstrated that CCL-5
17 (RANTES, a type I and III-regulated chemokine) production was impaired following
18 SeV *Cantell* infection in La-depleted cells (Figure 3a). Unsurprisingly, given that ISG
19 expression was only induced at mRNA level following 48 hpi, no CCL-5 release was
20 detected following infection with rSeV/eGFP strain across the 48 hour time course of
21 infection, nor was any significant difference observed in La-depleted cells, compared
22 with scrambled controls (Figure 3c). IFN- λ 1 release was completely abrogated in La-
23 depleted cells, compared with controls, following infection with either SeV strain,
24 further supporting our findings suggesting that La is crucial for the IFN response
25 downstream of viral infection (Figure 3b and d). Assessing the effect of La
26 knockdown on proinflammatory cytokine production in response to SeV *Cantell*
27 infection demonstrated that La knockdown had little or no effect on the ability of the
28 *Cantell* strain to induce IL-8, IL-6 or TNF- α (Figure 3e-g). Importantly, our results
29 demonstrate not only that La positively regulates type I IFN responses downstream
30 of SeV infection, but that it also has a novel role in promoting type III IFN induction
31 downstream of SeV infection.

32 ***La enhances RIG-I binding to RNA ligand via direct interaction with the CARD-***
33 ***domain of RIG-I***

1 SeV *Cantell* has been reported to rely entirely on RIG-I to elicit an anti-viral immune
2 response⁵⁵. Having demonstrated that La is required for type I and III IFN production
3 in response to SeV challenge, and given its ability to bind RNA, we hypothesised
4 that La may directly regulate RIG-I activation through regulation of RNA binding. To
5 test this hypothesis, HEK 293T cells were transfected with FLAG-tagged RIG-I and
6 increasing concentrations of La from 0-2 µg. Cell lysates were incubated with 1 µg
7 biotin-labelled poly(I:C), and poly(I:C)-binding proteins were subsequently isolated.
8 The ability of RIG-I to bind poly(I:C) was determined by western blotting using anti-
9 FLAG antibody. As Figure 4a shows, increasing concentrations of La enhanced the
10 ability of RIG-I to bind poly(I:C). A faint lower band on the gel (corresponding to La)
11 indicates, as would be expected from previous reports, that La was also capable of
12 direct interaction with the RNA ligand. The lower panel of Figure 4a demonstrates
13 total RIG-I and La expression in the lysates; endogenous La can be observed
14 strongly in the lane without FLAG-tagged La overexpressed, due to blotting with anti-
15 La antibody. However, transfection with increasing concentrations of La (lanes 2-5)
16 dose-dependently increases expression, as expected. Full blots as well as statistical
17 analysis of corresponding optical densitometry across three individual experiments
18 are shown in Supplemental Figure 2.

19 Having observed enhanced binding between RIG-I and its RNA ligand in the
20 presence of over-expressed La, we hypothesised that La may achieve this through
21 direct binding to RIG-I. Co-immunoprecipitation studies demonstrated an inducible
22 interaction between La and RIG-I following stimulation of RIG-I overexpressing HEK
23 293T cells with the RIG-I agonist, 5'ppp-dsRNA (Figure 4b, Supplemental Figure 3).
24 This inducible interaction was statistically significant across three independent
25 experiments (Supplemental Figure 3d). In addition, HeLa cells over-expressing GFP-
26 La and flag-tagged RIG-I were stimulated with 5'ppp-dsRNA, which induces La
27 translocation from the nucleus to the cytoplasm where it can co-localise and interact
28 with RIG-I (Figure 4c). Additional confocal images demonstrating this pattern are
29 shown in Supplemental Figure 4 and overlap coefficients for each image are given in
30 Supplemental Table S4.1. The translocation of La from the nucleus to the cytoplasm
31 is consistent with data from previous studies that demonstrate similar translocation of
32 La following viral infection^{44,45,56}. In addition, cells depleted of La had a reduced

1 response to 5'ppp-dsRNA in their ability to induce IFN- β , confirming the ability of La
2 to directly promote RIG-I induced IFN- β expression (Supplemental Figure 3c).

3 In order to determine the domains responsible for this interaction, either full-
4 length or N-terminal-only (aa 1-204) His-tagged recombinant La was incubated with
5 lysates from HEK 293T cells over-expressing full-length RIG-I, the CARD domain-
6 only of RIG-I, or the helicase domain-only of RIG-I. Potential interactions were
7 analysed by western blotting. As Figure 4d (upper panel) demonstrates, an
8 interaction was observed between full-length La and full-length RIG-I, as expected,
9 but also with the RIG-I deletion mutant expressing only the CARDs. No interaction
10 was observed with helicase-only mutant of RIG-I. This indicates that the activatory
11 CARD domains (spanning amino acids 1-284) of RIG-I are necessary and sufficient
12 for the interaction between La and RIG-I to occur. Further analysis demonstrated
13 that the N-terminal but not the C-terminal domain of La is required for interaction with
14 both full length and CARD domains of RIG-I (Figure 4d, lower panel).

15 Collectively, our results indicate a novel role for La as a positive regulator of type
16 I and type III IFN production in response to SeV infection. We demonstrate that the
17 mechanism of this regulation occurs through a direct interaction between La and
18 RIG-I, which promotes RIG-I binding to its cognate ligand. Our findings not only
19 contribute to the understanding of molecular mechanisms behind RIG-I-mediated
20 regulation of IFN induction, but also provide valuable insight into the potential that
21 dysregulation of La activity may contribute to over-activation of RIG-I and hence
22 dysregulated IFN production, as observed in autoimmune diseases such as SLE.

1 **Discussion:**

2 The induction of IFN expression is a crucially important part of the innate anti-viral
3 immune response, not only for destruction of viral RNA and limitation of viral spread,
4 but also for activation of adaptive immunity and selective killing of virally-infected
5 host cells. With this work we have demonstrated a novel interaction between La and
6 RIG-I, which results in enhanced RIG-I-RNA association. Knockdown of La resulted
7 in increased Sendai viral infection efficiency, decreased IFN- β , IFN- λ 1 and ISG
8 mRNA expression and attenuated CCL-5 and IFN- λ 1 release, compared with control
9 cells. Overall, these findings highlight an essential and novel role for La in mediating
10 optimal type I and type III IFN responses following viral challenge in order to protect
11 the host by both limiting viral replication and promoting the clearance of the
12 pathogen.

13 Type I IFNs are the first line of defence against most types of viral infection,
14 including the murine pathogen, SeV (DI⁺). They induce an anti-viral state in host
15 cells. This is achieved by JAK/STAT pathway-mediated activation of interferon-
16 stimulated genes (ISGs), such as RIG-I, CXCL-10, CXCL-11, OAS1 and OAS2⁵⁷.
17 Our findings demonstrated a significant decrease in the induction of IFN- β , CXCL-10,
18 and CXCL-11 following SeV infection upon depletion of La. While type III IFNs are
19 structurally and genetically distinctive from type I IFNs and act through a separate
20 receptor system, they have similar mechanisms of induction, signal transduction and
21 biological function^{26,58}. SeV is a potent inducer of type III IFN responses^{27,59}. Our
22 study identifies La as a novel positive regulator of IFN- λ 1 induction downstream of
23 SeV infection. Collectively our results indicate an important role for La in inhibiting
24 SeV replication by promoting both type I (as seen following *Cantell* infection) and
25 type III (as seen following *Cantell* and rSeV/eGFP) IFN responses.

26 RIG-I is central to the regulation of both type I and type III IFN production as it
27 is responsible for detection of SeV infection within cells. Regarding regulation of
28 RIG-I activity, a number of proteins have been identified to play a role through post-
29 translational modifications. For example, TRIM25 and Riplet/RNF135/REUL induce
30 K63-linked ubiquitination within the CARD domains of RIG-I following viral infection,
31 a modification which is necessary for interaction with IPS-1⁶⁰⁻⁶². In addition, CK2-
32 mediated phosphorylation of RIG-I at Thr 770 and Ser 854 inhibits the anti-viral

1 response to both hepatitis C virus and SeV and renders RIG-I inactive⁶³. This
2 prevents TRIM25-mediated ubiquitination of RIG-I, thereby negatively regulating the
3 IFN response⁶⁰. With this work, we have identified a novel function for the
4 autoantigen La in enhancement of anti-viral responses. Specifically, it binds directly
5 to the RIG-I receptor in an inducible manner and strengthens RIG-I binding to its
6 RNA ligand, making it unique in its mechanism of action from other known RIG-I
7 regulators, such as TRIM25 and CK2. Thus La positively regulates type I and type III
8 IFN responses by augmenting stable RNA-RIG-I complex formation, which results in
9 robust pathway activation. As RIG-I can also drive inflammatory gene expression
10 through interaction with a IPS-1-CARD9-Bcl-10 complex and activation of NF κ B⁶⁴, it
11 would appear that the interaction between La and RIG-I is able to enhance the IFN- β
12 response (presumably via enhancing interaction of IPS-1 with TBK-1) possibly
13 independent of the ability of RIG-I to drive NF κ B activation. This is supported by the
14 fact that La knockdown has no effect on inflammatory gene expression downstream
15 of RIG-I interaction whereas IFN- β expression is severely reduced.

16 Regarding a potential role for La in regulating assembly of RNA-binding complexes,
17 Liu and colleagues⁴⁶ demonstrated a role for La in RNAi processing. They reported
18 that La associated with Ago2 of the RISC complex in an RNA-dependent manner,
19 thereby promoting RISC complex catalysis and RNAi processing. This finding is
20 consistent with our data which show that La augments RIG-I binding to poly I:C.
21 Importantly, deletion of the RNA-binding domain of La blocks interaction with RIG-I,
22 underlining the RNA-binding role of La in driving RIG-I activity. In keeping with our
23 findings that La is a positive regulator of viral-induced type I IFN, Liu *et al* showed
24 that La could promote the anti-viral response to *flock house* virus (FHV) in
25 *Drosophila* S2 cells. Indeed, La depletion resulted in increased FHV infectivity,
26 which supports our findings that La promotes anti-viral responses to Sendai virus in
27 HEK 293T cells. In contrast, Bitko and colleagues demonstrated enhanced IFN- β
28 mRNA levels and decreased viral titres upon siRNA depletion of La⁴³. In addition,
29 Domitrovich *et al.* argued a role for La as a negative regulator of IFN production in
30 the context of HCV replication, based on a 63-67% reduction in RNA replication in
31 the absence of La in Huh7 cells and increased IFN- β mRNA 10 hours post-RSV
32 infection in the absence of La⁴⁴. However, similar to our findings, both of these
33 studies also observed an overall decrease in IFN- β production 24 hours post-

1 infection in La-depleted cells, suggesting that La may play a dual role in regulating
2 anti-viral responses. Indeed, La may play a role in maintaining homeostasis in cells
3 with respect to IFN- β production, as evidenced by the enhanced IFN- β expression in
4 unstimulated cells depleted of La (Supplementary Figure 3c), whereas when RIG-I is
5 activated, depletion of La results in substantial reduction in IFN- β production. This
6 indicates that the loss of La may somehow disrupt homeostatic mechanisms to
7 maintain appropriate IFN- β levels or indeed may inhibit viral-specific evasion
8 mechanisms. For example, the RSV-derived NS2 protein binds to RIG-I and blocks
9 its interaction with IPS-1, thereby preventing IRF-3 activation^{65,66}. Thus the loss of
10 La may disrupt the negative function of NS2 on this system, thereby contributing to
11 the enhanced IFN- β observed in similar studies. Extensive studies would be required
12 to address these questions, which are outside the scope of this manuscript.

13 With this work, we identify role for La in regulating IFN responses by promoting
14 the RIG-I-mediated anti-viral response through direct association with RIG-I and
15 enhancing RIG-I binding to its viral agonist. Importantly, our study is the first to
16 assess the role for La in regulating type III IFN responses, with all previous studies
17 focusing on type I IFN only. SeV infection experiments support these findings, with
18 depletion of La resulting in increased viral infectivity and decreased type I and type
19 III IFN responses, compared with controls. These findings highlight an important and
20 novel role for La in the promotion of optimal type I and type III IFN responses
21 following SeV challenge, serving to protect the host through limiting viral replication.

22

23

1 **Methods**

2 **Materials**

3 The SW5 monoclonal La antibody was generated by Professor Michael Bachmann
4 at the Technical University of Dresden and was a kind gift from Dr. JS Maier-Moore
5 ⁶⁷. All flag-tagged RIG-I plasmid constructs were a kind gift from Dr. Kate Fitzgerald
6 (UMASS Med School, Worcester, MA). The GFP-tagged La construct was a gift from
7 Dr. Karl Albert Brokstad (University of Bergen, Germany). Monoclonal M2 Flag
8 antibody was purchased from Santa Cruz, pcDNA3.1 empty vector control from
9 Invitrogen and biotin-labelled poly(I:C) from Cayla-Invivogen. A Mission® shRNA
10 construct specific to human La, as well as a scrambled control, were purchased from
11 Sigma.

12

13 **Cell Culture**

14 HEK 293T and HeLa cell lines were cultured in Dulbecco's Modified Essential
15 Medium (DMEM) containing stable 2 mM L-glutamine, 10% (v/v) foetal calf serum
16 (FCS), 100 units/ml Penicillin, 100 µg/ml Streptomycin and 100 µg/ml gentamicin.
17 LLC-MK2 cells (ECACC 85062804) were grown in minimum Eagle's medium (MEM)
18 containing 40 g/ml non-essential amino acids and supplemented with 10% heat
19 inactivated FCS, 2 mM L-glutamine, 2 mg/ml sodium carbonate, 100 g/ml gentamicin
20 and 1.25 mg/ml Fungizone. Cells were maintained at 37°C in a humidified
21 atmosphere of 5% CO₂.

22

23 **La Knockdown**

24 HEK 293T cells were seeded at 5×10^4 cells per ml and transfected the following
25 day with 500 ng of scrambled or La-specific shRNA (Sigma). Following 48 hr, cells
26 were washed with PBS prior to viral infection as detailed below.

27

28 **Viral Infection**

29 The rescue and characterisation of recombinant Sendai virus expressing eGFP
30 (rSeV/eGFP) was previously described ⁶⁸. The SeV Cantell strain, a wild type strain
31 containing defective interfering particles, comes originally from Charles River
32 Laboratories. Media was discarded and replaced with fresh pre-warmed DMEM
33 supplemented with antibiotic only (no FCS), in order to limit cell growth. Cells were

1 then infected with either rSeV/eGFP or SeV *Cantell* at a multiplicity of infection (MOI)
2 of 0.1 or 10, as indicated in figure legends. One hour post-infection, inocula were
3 removed by discarding media and replaced with DMEM supplemented with antibiotic
4 and 1% FCS to ensure cell survival while maintaining limited growth. At indicated
5 time points, media was carefully removed and retained for subsequent cytokine
6 analysis, cells were gently re-suspended in ice-cold PBS and centrifugation was
7 carried out at 400×g for 5 min to pellet cells for subsequent analysis. Cells were then
8 re-suspended in Trizol reagent for gene expression analysis or SDS sample buffer
9 supplemented for protein expression analysis.

11 ***SeV titrations***

12 For SeV/eGFP, a 1:10 dilution series of the sample was added to LLC-MK2 cells in
13 MEM 1% FBS. At 24 hpi fluorescent foci were counted. The titer is calculated as
14 fluorescent forming units (FFU) by multiplying the average number of foci by the
15 dilution factor at a given dilution. The dilution at which the foci were counted is equal
16 to the inverse of the exponent of the final FFU. The titer of SeV Cantell stock was
17 determined by plaque assay as previously described⁶⁹.

19 ***Real-time polymerase chain reaction (qPCR)***

20 RNA was extracted from cell cultures using Trizol™ (Sigma) and reverse transcribed
21 to complementary DNA using the GoScript Reverse Transcription kit (Promega), as
22 per manufacturer's instructions. Real-time quantitative PCR investigating gene
23 expression was performed using primers listed in Table 1, with SYBR Green Taq
24 ReadyMix (Sigma) according to manufacturer's recommendations. Data were
25 analyzed using an ABI Prism 7900 system (Applied Biosystems) and were
26 normalized to 18s RNA. Real-time PCR data were analyzed using the $2^{-\Delta\Delta Ct}$ method⁷⁰.

29 ***Western blotting***

1 To prepare whole cell lysates, cells were lysed in SDS buffer (250 mM Tris-HCl, pH
2 6.8, 10% SDS, 0.5% Bromophenol blue, 50% Glycerol, 50 nM DTT) and boiled at
3 95°C for 10 min. Equal quantities of whole cell lysates were resolved by
4 electrophoresis on a denaturing SDS–polyacrylamide gel according to the method of
5 Laemmli ⁷¹ and transferred to a nitrocellulose membrane. Following immunoblotting,
6 the membrane was developed using enhanced chemiluminescent horse radish
7 peroxidase (HRP) substrate (Millipore).

9 ***Co-immunoprecipitation***

10 Cells were treated as described in the figure legends, lysed in EBC lysis buffer
11 (Deionised water containing 50 mM Tris (pH 8.0), 150 mM NaCl, 1% Nonidet P40,
12 0.5% (w/v) sodium deoxycholate and 0.1% SDS containing 1 mM Sodium
13 orthovanadate (Na₃VO₄), 1 mM Phenylmethylsulfonylfluoride (PMSF), 1 mM
14 Potassium fluoride (KF),) and incubated with SW5 anti-La antibody coupled to
15 protein A sepharose beads. Thereafter, immune complexes were washed and re-
16 suspended in SDS sample buffer for western blot analysis.

18 ***Recombinant protein pull-downs***

19 Following a gentle wash with ice-cold PBS, lysates were prepared by addition of
20 EBC buffer. After sonication and centrifugation, the supernatant was incubated with
21 50 µl Nickel agarose beads coupled to approximately 1 µg either full length (8A) or
22 N-terminal truncated (7A) recombinant La (Dr. J. Maier-Moore), for 2 h on rotation at
23 4°C. After incubation, nickel agarose was washed three times with EBC buffer by
24 gentle inversion and centrifugation at 5,000 × g. Beads were then re-suspended in
25 SDS sample buffer for western blot analysis.

27 ***Enzyme-linked Immunosorbance Assay (ELISA)***

1 ELISAs were carried out using DuoSet® ELISA Development Kit for human CCL-5
2 (Rantes) or human IFN- λ 1 (IL-29) (eBioscience) as per the manufacturer's
3 instructions.

5 ***RNA Immunoprecipitation***

6 Cells were seeded and transfected as indicated in figure legends. Cells were lysed
7 for 20 min at 4°C on rotation in freshly prepared sterile RNA Immunoprecipitation
8 (RIP) buffer (150 mM KCl, 25 mM Tris pH 7.4, 5 mM EDTA, 0.5 mM DTT, 0.5% NP-
9 40) supplemented with protease inhibitors and SUPERase RNase inhibitor (Sigma).
10 Samples were sonicated for 15 sec, cell debris was pelleted by centrifugation and
11 cell supernatants were transferred to fresh tubes. One μ g of biotin-labelled poly(I:C)
12 (Invivogen) was added, followed by incubation at 4°C for 1-2 h. The samples were
13 then added to 50 μ l of pre-washed UltraLink NeutrAvidin beads (Pierce) and
14 incubated for 1 h at 4°C. The resulting immune complexes were then washed with
15 RIPA buffer and re-suspended in 30 μ l SDS sample buffer, prior to western blot
16 analysis.

18 ***Immunofluorescence***

19 HeLa cells were seeded at 1×10^5 /well on coverslips, transfected and stimulated as
20 indicated in figure legends. Cells were fixed and permeabilised with 4%
21 paraformaldehyde and 0.2% (v/v) Triton X-100 in PBS. After washing, cells were
22 blocked in PBS with 1.2% (w/v) Fish Gelatin and 100 mM glycine and then incubated
23 at 37°C for 1 h with the primary antibody of interest at 1:100 dilution in blocking
24 buffer, followed by detection with the appropriate fluorescently labelled secondary
25 antibody at 1:200 dilution. Cells were mounted and nuclei stained using ProLong®
26 Gold anti-fade reagent with DAPI. Cells were imaged using the LSM 710 System
27 (Carl Zeiss) and analysed for co-localisation using Zen 9 software.

29 ***Statistical analysis***

1 All data was analysed using GraphPad Prism (version 7) statistical software
2 package, as specified. Statistical comparison between groups was carried out using
3 tests described in figure legends. Data is graphically represented as mean +/-
4 standard error of the mean (SEM). *P* values less than or equal to 0.05 were
5 considered significant.

6

7 ***Data Availability***

- 8 • No datasets were generated or analysed during the current study.
- 9 • All data generated or analysed during this study are included in this published
10 article (and its Supplementary files).

11

12 **Acknowledgements**

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14 Albert Brokstad for plasmid constructs.

15

16 **Author Contributions**

17 RM was responsible for study design, data collection, data analysis and drafting of
18 the manuscript. LB was responsible for data collection, data analysis and manuscript
19 revision. UFP was involved in the coordination of the project, data analysis and
20 critical revision of the manuscript. JMM supplied reagents, helpful discussion and
21 critical review of the manuscript. CAJ was responsible for initial conception and
22 design of the study, data analysis and critical revision of the manuscript.

23

24 **Additional information**

25 The authors declare no competing financial interests.

26

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3 4 **Figure legends**

5 **Figure 1: Depletion of La in HEK 293T results in an increase in Sendai viral**
6 **infection efficiency.** HEK 293T cells were transfected with 500 ng of La-specific or
7 scrambled Mission® shRNA (Sigma) for 48 h. Cells were then infected with
8 rSeV/eGFP at an MOI of 0.1. (a) GFP positivity was visualised using a Nikon Eclipse
9 TE2000-U and a Hamamatsu ORCA ER camera. (b) % monolayer GFP-positive
10 analysis was carried out using Image J software. (c) rSeV/eGFP titrations (FFU/mL)
11 were carried out on LLC-MK2 cells. Areas under the curves were calculated and
12 compared. Results are from two independent experiments carried out in duplicate.
13 (d) 48h post transfection, a well from each condition (Scrambled or La) was
14 trypsinised, following which a trypan blue cell count was performed to determine the
15 number of viable cells prior to infection. Data shown is combined average cell counts
16 from two independent experiments.

17
18 **Figure 2: IFN- β IFN- λ and ISG mRNA expression is attenuated in La-depleted**
19 **cells following SeV infection.** HEK 293T cells were transfected with 500 ng of
20 either La-specific or scrambled Mission® shRNA (Sigma) for 48 h after which they
21 were infected with SeV *Cantell* (a-d) or rSeV/eGFP (e-h) at an MOI of 10 and
22 incubated for the indicated time points. IFN- β (a, e), IFN- λ 1 (b, f), CXCL11 (c, g),
23 CXCL10 (d, h) expression was determined by RT-qPCR. Data shown are a
24 representative of three independent experiments in each case. * $p < 0.05$, ** $p < 0.01$,
25 *** $p < 0.001$ and **** $p < 0.0001$, as determined by unpaired *t*-test, comparing scrambled
26 to La shRNA at each time point.

27
28 **Figure 3: CCL5 & IFN- λ 1 release is decreased in La-depleted cells following**
29 **SeV infection.** HEK 293T cells were transfected with 500 ng of either La-specific or
30 scrambled Mission® shRNA (Sigma) for 48 h after which they were infected with
31 SeV *Cantell* (a-b) or rSeV/eGFP (c-d) at an MOI of 10. CCL5 (a, c) and IFN- λ 1 (b, d)

cytokine release was determined by ELISA. IL-8 (e), IL-6 (f) and TNF- α (g) was measured from cells supernatants using a multi-plex human pro-inflammatory 7-spot assay (MSD). Data shown is the combined average of three independent experiments. * $p < 0.05$, ** $p < 0.01$ and **** $p < 0.0001$, as determined by unpaired t -tests, comparing scrambled to La shRNA at each time point.

Figure 4: La enhances RIG-I binding to RNA ligand and interacts with RIG-I following 5'ppp-dsRNA stimulation. (a) HEK 293T cells were transfected with 4 μ g EV or 2 μ g FLAG-tagged RIG-I with increasing FLAG-tagged La, as indicated. Analysis of the ability of FLAG-tagged La or RIG-I to bind biotinylated poly(I:C) was assessed by western blotting with anti-FLAG antibody. Expression of FLAG-tagged or RIG-I constructs in whole cell lysates was determined by western blotting with either anti-La or anti-RIG-I antibodies, as appropriate. (b) HEK 293T cells were transfected as indicated and stimulated for 1, 3, or 6 h with 1 μ g 5'ppp-dsRNA (Invivogen). Following immunoprecipitation of La-containing complexes with a La-specific antibody, the ability of over-expressed RIG-I to interact with endogenous La was determined by western blotting using anti-FLAG antibody. (c) HeLa cells were seeded on UV-irradiated coverslips, transfected with 2 μ g of GFP-tagged La and 2 μ g FLAG-tagged RIG-I, following which they were stimulated with 1 μ g 5'ppp-dsRNA for 6 h. Immunostaining with anti-RIG-I antibody indicates that La and RIG-I co-localise following stimulation with 5'ppp-dsRNA. Both images are at 63X magnification. (d) Recombinant La was incubated with lysates prepared from HEK 293T cells overexpressing FLAG-tagged RIG-I, FLAG-tagged RIG-I-CARD, or FLAG-tagged RIG-I-Helicase (Heli), as indicated. The ability of RIG-I and La to interact was analysed by western blotting. In all cases images are representative of three independent experiments.

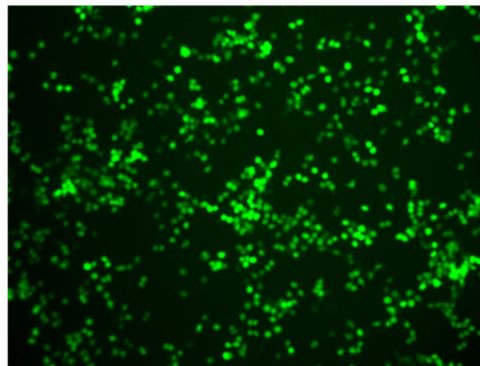
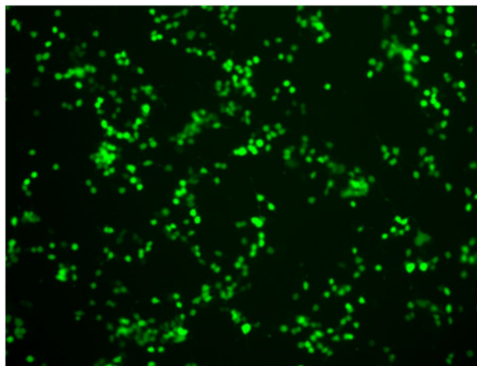
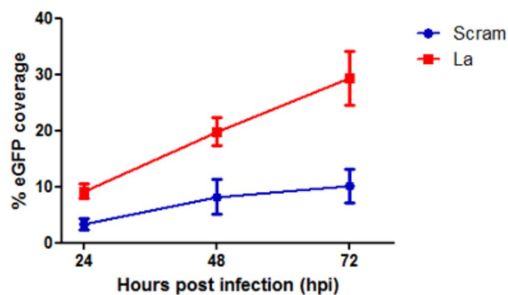
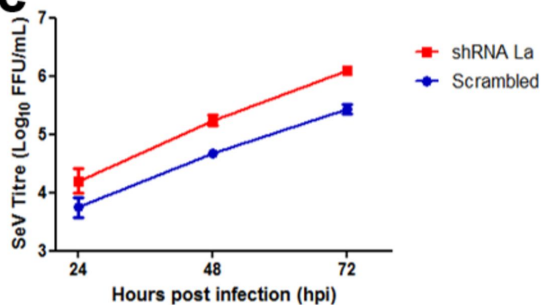
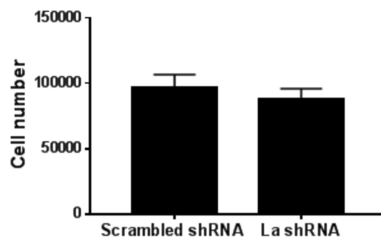
Table 1: Human primers used in this study;

Primer Name	Primer Sequence	Product Size(bp)
La sense	GAAGGAGAGGTGGAAAAAG	372
La anti-sense	AAGCCCCGCAACAAAAG	
IFN- β sense	CTAGCACTGGCTGGAATGAGA	217

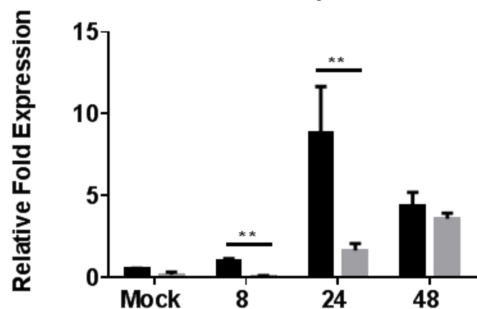
IFN- β anti-sense	CTGACTATGGTCCAGGCACA	
18S sense	TTGACGGAAGGGCACCACCA	131
18S anti-sense	GCACCACCACCCACGGAATCG	
IFN- λ 1 sense	GGACGCCTTGGAAGAGTCACT	84
IFN- λ 1 anti-sense	AGAAGCCTCAGGTCCCAATTC	
CXCL-10 sense	GGAAGCACTGCATCGATTTTG	519
CXCL-10 anti-sense	CAGAATCGAAGGCCATCAAGA	
CXCL-11 sense	GCCTTGGCTGTGATATTGTGTG	686
CXCL-11 anti-sense	CACTTTCACCTGCTTTTACCCCAG	

1

2

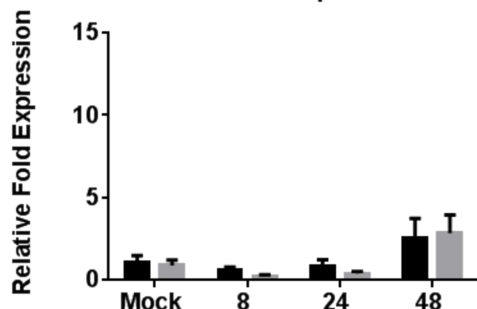
a**La knockout****Scrambled****b****SeV eGFP MOI:0.1****c****d****HEK 293T cell viability**

IFN- β



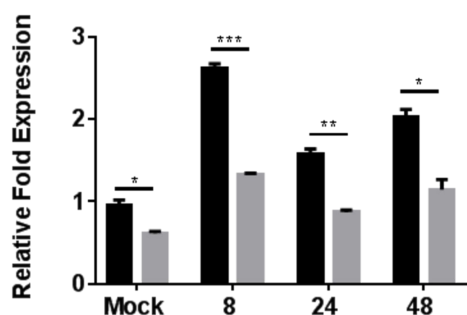
SeV Cantell h.p.i.

IFN- β



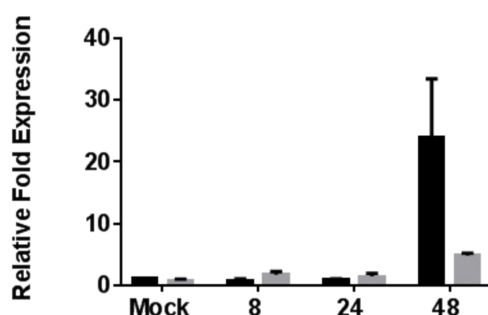
SeV eGFP h.p.i.

IFN- λ 1



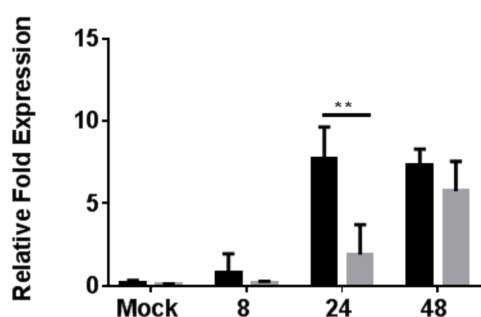
SeV Cantell h.p.i.

IFN- λ 1



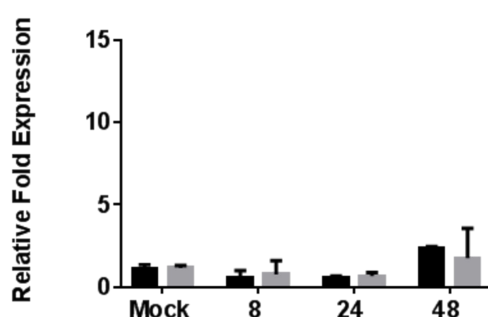
SeV eGFP h.p.i.

CXCL-11



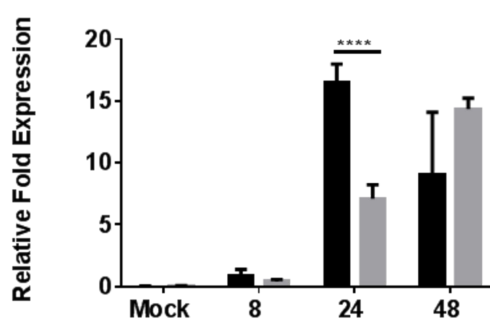
SeV Cantell h.p.i.

CXCL-11



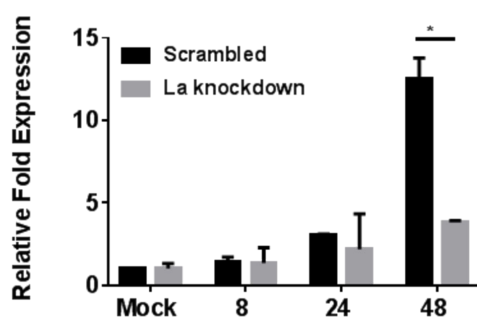
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CXCL-10



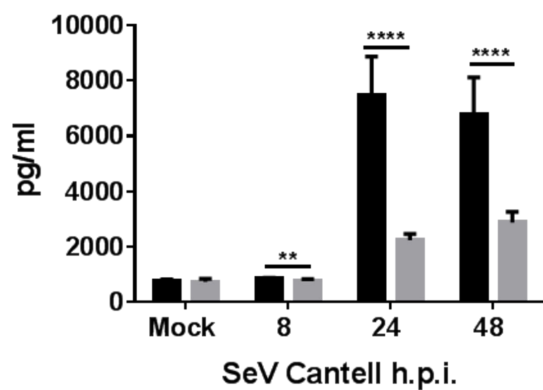
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CXCL-10

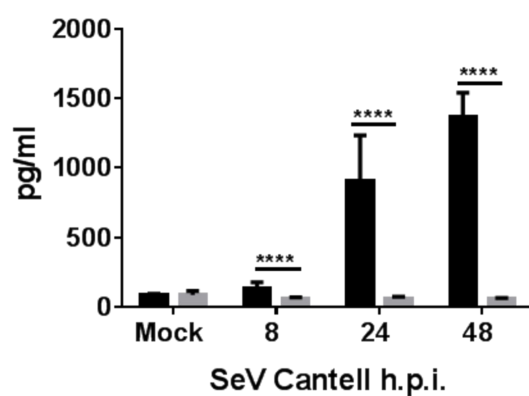


SeV eGFP h.p.i.

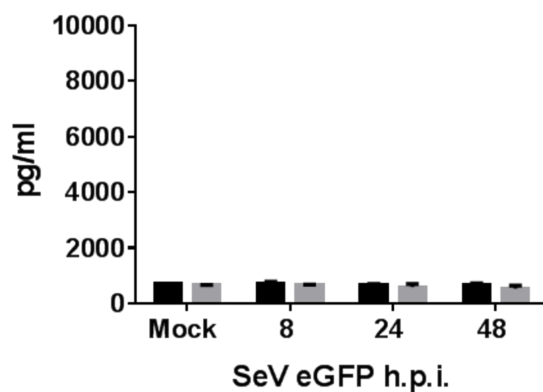
CCL-5



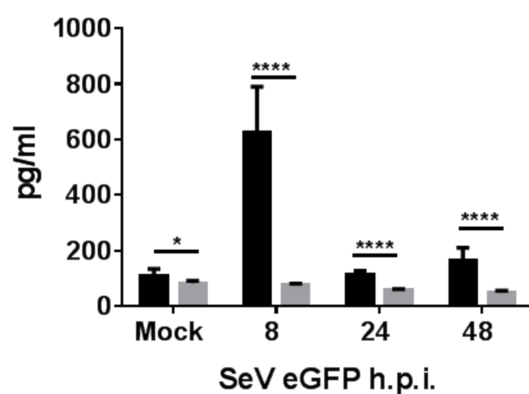
IFN- λ 1



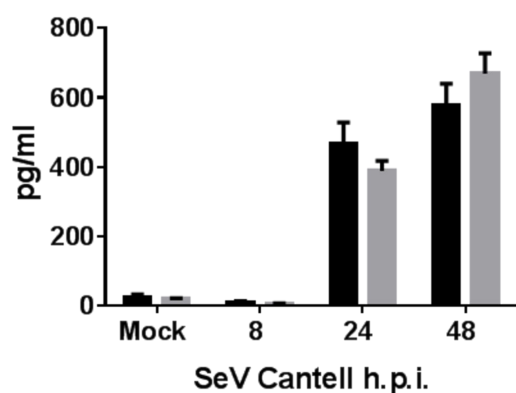
CCL-5



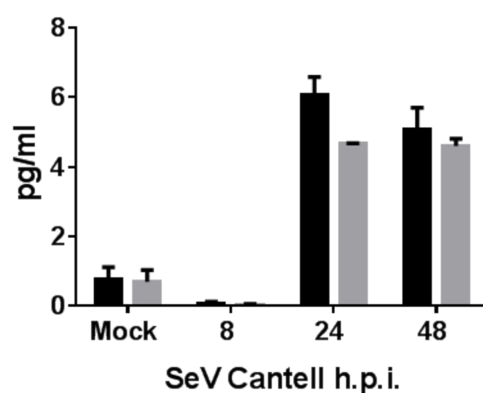
IFN- λ 1



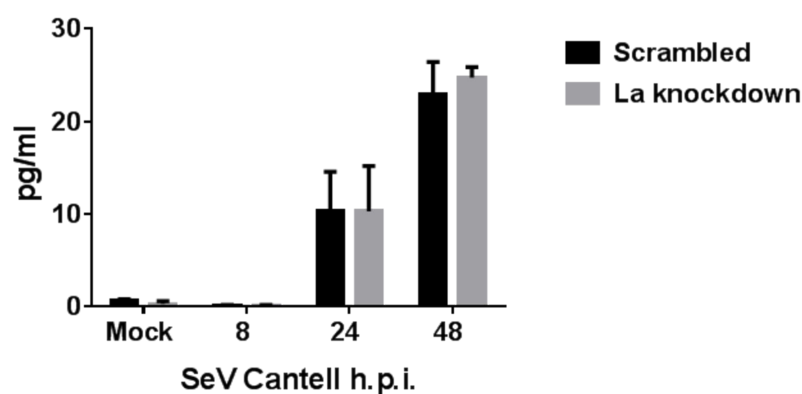
IL-8

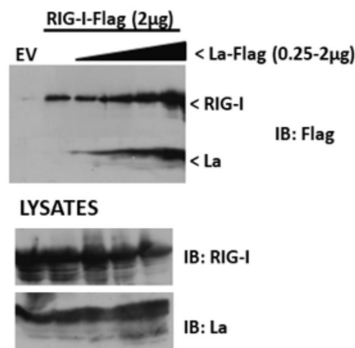
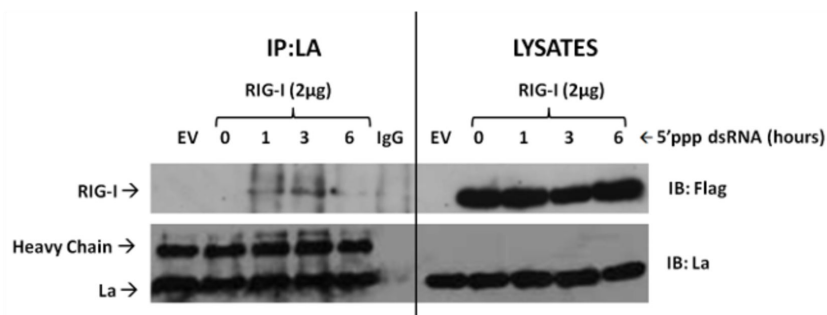
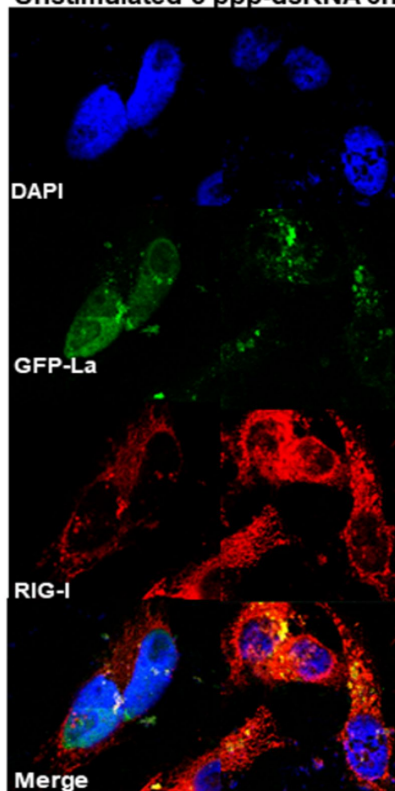


IL-6



TNF- α



a**POLY (I:C) PULLDOWN****b****c****Unstimulated 5'ppp-dsRNA 6hr****d**